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Reprinted from THE JOURNAL OF PATHOLOGY AND BACTERIOLOGY,
Vol. XXIX., 1926, pp. 118-122.

COMBINED SCHICK TEST AND DIPHTHERIA PROPHYLACTIC; COMBINED DIPHTHERIA-SCARLET-FEVER PROPHYLACTIC.

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Combined Schick test and diphtheria prophylactic.

THE Schick dose of toxin has a small immunising effect in animals already immune but its antigenic value as a primary stimulus is negligible. A preparation that would give a satisfactory Schick reaction and at the same time act as an immunising agent would have a distinct advantage as it would reduce the number of subsequent prophylactic injections for human beings from 3 to 2. It would also act as the commencement of a second course of immunisation in the small number of children who, when tested after a first series of inoculations, prove to be still positive to the Schick test. If such a preparation can be made of equal antigenic value to the diphtheria immunising agents already in use it might displace them entirely, for each injection would record the stage of immunisation reached. Such a diagnostic and prophylactic mixture (D.P.M.) must contain a relatively large amount of toxin or toxoid so masked with antitoxin that the total toxin free is the same as that in Schick toxin in order to produce corresponding reactions, and any excess toxoid must be in such a form that its presence will not interfere with the combining power of the toxin; thus the mixture will still detect the same level of immunity as the ordinary Schick dose of toxin.

For some years we have been endeavouring to produce a satisfactory mixture; at first we hoped to be able so to modify toxoid that its affinity for antitoxin would be far less than that of toxin and that its addition to Schick toxin would not interfere with the combination of the toxin with any antitoxin presented to it. If such a mixture were injected into an animal with a small degree of immunity the antitoxin present in the blood would neutralise the same amount of toxin in the mixture as it would in a Schick dose of toxin without combining with the toxoid of weaker affinity; this would then be free to act as an immunising agent. These experiments have been unsuccessful but preliminary work on a mixture consisting of a suspension in ordinary Schick toxin of toxoid precipitated by alum are giving promising results.

The diagnostic and prophylactic mixtures described in this paper consist of toxin partially neutralised by antitoxin and diluted until the amount of toxin free was the same as in Schick toxin. It was necessary to test many batches of toxin before a suitable one could be found. The ratio of toxin to toxoid and consequently the ratio of toxin to combining power in a partially neutralised toxin is always less than in the original toxin. The Schick test dose (or our desired double Schick test and mixture) must contain a fixed amount of free toxin and must possess a definite combining power. Too little free toxin will lead to indefinite reactions; too much toxin would cause too severe reactions in the non-immune. The combining power must be fixed so that a constant level of immunity is detected by the presence or absence of a reaction. The following tests serve to show that the diagnostic value of the diagnostic prophylactic mixture (DPM) is approximately the same as Schick toxin.

Table I. gives the average of a series of tests upon the minimal reacting dose to determine the amount of toxin free and a combining-power test indicating the total amount of toxin and toxoid free. The same table gives a typical series of tests for Schick toxin.

TABLE I.

Comparison between DPM 1 *and Schick toxin.*

M.R.D. Reactions produced by the intracutaneous injection into guinea-pigs of 0·2 c.c. of various dilutions.			Combining power. Reactions produced by the intracutaneous injection into guinea-pigs of 0·2 c.c. of mixtures of equal parts of toxin or D.P.M. with dilutions of antitoxin containing various fractions of a unit per c.c.		
Antitoxin.	D.P.M.	Schick.	Antitoxin.	D.P.M.	Schick.
1/30	–	–	1/150	–	–
1/25	s	s	1/200	±	s
1/20	±	+	1/250	+	+
1/15	+	+	1/300	+	+
1/10	+	+	1/400	+	+

Table II. shows the reactions produced by the DPM 1 side by side with varying strengths of Schick toxin in passively immunised guinea-pigs. Guinea-pigs of different weights were injected subcutaneously with ½, 1 and 2 units of antitoxin and tested on the following day and in some cases on several successive days. It will be seen that the DPM 1 appeared from this test to be slightly stronger than the Schick toxin but less than double the strength.

TABLE II.

Comparison between DPM 1 *and Schick toxin.*

(Passively immune guinea-pigs.)

Antitoxin injected.	Tested.	Schick doses.			DPM 1.
		½	1.	2.	
0·5 unit	1 day after	–	+	+	+
	2 days after	–	s	+	+
	3 ,,	s	±	+	+
	4 ,,	±	+	+	+
1·0 unit	1 day after	–	–	–	s
	2 days after	–	–	–	–
	3 ,,	–	s	±	s
	4 ,,	–	–	+	±
0·5 unit	1 day after	–	–	+	–
1·0 ,,	1 ,,	–	–	+	–
2·0 units	1 ,,	–	–	+	–
0·5 unit	1 day after	–	–	±	±
1·0 ,,	1 ,,	–	–	+	+
2·0 units	1 ,,	–	–	±	–

A further series of guinea-pigs recorded in table III. were actively immunised with a small dose of toxoid; two weeks later we commenced weekly injections of both DPM 1 and Schick toxin. In these experiments both diagnostic agents detected the presence of acquired immunity at the same time.

Table III.

Comparison between DPM 1 *and Schick toxin.*

(Actively immune guinea-pigs.)

Weeks after receiving 1/4 Lf toxoid.	Reactions produced by Schick toxin and by DPM in six guinea-pigs.											
	S	D	S	D	S	D	S	D	S	D	S	D
2	+ +	+ +	+ +	+ +	+ +	+ +	+ +	+ +	+ +	+ +	+ +	+ +
3	+ +	+ +	+ +	+ +	+ +	+ +	+ +	+ +	+ +	+ +	+ +	+ +
4	+ +	±	+	S	S	S	–	–	S	–	–	–
5	–	–	–	–	–	–	–	–	–	–	–	–
	S records the reactionproduced by the Schick toxin. D records the reaction produced by D.P.M.											

Experiments were then made to show the immunising value of DPM 1. A series of guinea-pigs received an intracutaneous injection of 0·2 c.c. of Schick toxin or DPM 1 followed one and two weeks later with a single immunising dose of 5 c.c. toxoid. Ten days later they were all given a series of Schick tests every two days according to our usual "rapid immunity index test." It will be seen from table IV. that the guinea-pigs that had received DPM 1 a week earlier than the toxoic became more rapidly immune than those that were given a preliminary injection of Schick toxin and that when the interval was extended to two weeks three out of four of the animals receiving a first injection of DPM 1 were already immune when tested ten days after the injection of toxoid. Similar results are shown in table V. for guinea-pigs receiving a much smaller immunising dose of toxoid and tested according to our usual immunity index method by weekly injections of Schick toxin commencing three weeks after the injection of the toxoid.

Table IV.

Immunising effect of DPM 1 *in guinea-pigs.*

1st Injection . . .	Schick	DPM 1	Schick	DPM 1
Interval	1 week		2 weeks	
2nd Injection . . .	5·0 c.c. toxoid		5·0 c.c. toxoid	
Days before Schick negative	16, 16, 16, 16, 16, 30	12, 14, 14, 14, 14	19, 21	10, 10, 10, 12

Table V.

Immunising effect of DPM 1 *in guinea-pigs.*

1st Injection . . .	Schick	DPM 1	Schick	DPM 1
Interval	1 week		2 weeks	
2nd Injection . . .	0·2 c.c. toxoid		0·2 c.c. toxoid	
Immunity index . . .	3, 4, over 4, over 5, 5	2, 2, 2	3, 6, 6, 6	2, 3, 3, 6

The D.P.M. used in the above experiments contained only the approximate equivalent of 1/200th of a L + mixture in 0·2 c.c. This dilution was necessary in order to keep uncombined toxin and toxoid down to the Schick level. Such a D.P.M. contains only 1/20th of the total antigenic material present in the usual immunising mixtures consisting of 1/10th L+ T.A.M. and yet its value as a primary stimulus can be definitely demonstrated in guinea-pigs. By testing a number of other toxins we have now been able to produce a D.P.M. (= DPM 2) containing the equivalent of 1/10th L+ T.A.M. in 0·2 c.c. *i.e.* equivalent to the amount in 1 c.c. of ordinary prophylactic mixture T.A.M; it also corresponds in diagnostic value to Schick toxin. Tests on this material are as yet incomplete but work is being continued on mixtures of this type and on those consisting of precipitated toxoid suspended in Schick toxin. If the control could consist of an equivalent mixture made from the same toxin modified by formaldehyde with the same amount of antitoxin added, both the control and the test dose will act as antigens. Probably the chief difficulty to be faced will be the increased number of pseudo-reactions but this may be overcome by making the mixtures more dilute.

Combined diphtheria-scarlet-fever prophylactic.

Another possible improvement in connexion with immunisation may consist in combining in one mixture diphtheria and scarlet fever toxins or toxoids. We have recently made various combinations of these substances. Whether these will act as efficient double immunising agents against both diphtheria and scarlet fever remains to be seen.

A dose of 1 c.c. of a diphtheria-scarlatina prophylactic (=DSP) which contains, in 1 c.c., 0·16 c.c. of our routine scarlet fever toxin and 0·1 c.c. of a toxoid-antitoxin mixture, when injected into guinea-pigs gave an immunity index of 5 in two guinea-pigs and 6 in a third; 5 c.c. gave immunity indexes of 4 and 5. This index was very little if at all below that which would have been given by the toxoid-antitoxin mixture alone, *i.e.*, the addition of the scarlet fever toxin (=5000 minimal skin reacting doses per c.c.) had not materially depressed the diphtheria immunity index. Another mixture containing 0·9 c.c. of scarlet fever toxin (=30,000 minimal skin doses) and 0·1 c.c. of toxoid antitoxin mixture gave a very low immunity index, *i.e.*, over 7 in five out of six guinea-pigs. Thus an excess of scarlet fever toxin depresses the immunising effect of the diphtheria prophylactic but it is clear that a double mixture can be made which retains its efficiency as diphtheria prophylactic. It was necessary to settle this point before using such a mixture in human work; lengthy clinical trials alone can determine whether the double prophylactic is efficient also as an immunising agent against scarlet fever. Clinical trials are already in progress.

It may be possible in the future to make various mixtures of Schick toxin and scarlet fever toxoid or Dick toxin and diphtheria toxoid so that any desired combination of Dick and Schick test and double immunisation may be available.

Summary.

1. A diphtheria diagnostic prophylactic mixture (DPM) is described which in the usual intradermic dose of 0·2 c.c. indicates the patient's Schick reaction and also acts as the first immunising dose.

2. A double diphtheria-scarlet-fever prophylactic (DSP) is described in which the proportions of the two constituents are so arranged that the amount of scarlet fever toxin does not depress the immunising efficiency of the diphtheria toxoid present.

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